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(54) Recovery of carotenoids, tocopherols, tocotrienols and sterols from esterified palm oil

(57) The invention is for a method for the isolation of the minor non-glyceride components of palm oil or like vegetable oil containing free fatty acid and non-glyceride components similar to that of palm oil which method comprises:

- (i) esterifying the free fatty acid component of the oil with one or more monohydric alcohols to form an esterified oil with a very low free fatty acid content,
- (ii) converting the glycerides into monoesters by transesterification employing one or more monohydric alcohols,
- (iii) adsorbing the non-glyceride components onto a selective absorbent to separate said components from the esters of the oil, and
- (iv) thereafter desorbing the non-glyceride components from the adsorbent with the use of solvent to recover said components. The adsorbent is preferably activated alumina, activated carbon, or silica gel, preferably reverse phase (particularly C18) silica gel. By the method, carotenes, sterols, tocopherols and other non-glyceride components can be isolated.

RECOVERY OF CAROTENOIDS, TOCOPHEROLS, TOCOTRIENOLS
AND STEROLS FROM ESTERIFIED PALM OIL

Crude palm oil contains about 1% of non-glyceride components which include carotenoids, tocopherols, tocotrienols and sterols. The carotenoids, consisting of mainly α and β carotenes at 500 to 700 ppm, are important constituents with pro-vitamin A activity, possible anti-tumor formation properties, and other physiological activities. The tocopherols and tocotrienols are Vitamin E constituents and also natural anti-oxidants, and are present at approximately 600 to 1000 ppm in crude palm oil; the major component is the gamma-tocotrienol which has recently been found to have anti-cancer properties besides its known anti-oxidant activity. Tocotrienol has been found to lower blood cholesterol. (The sterols consists mainly of sitosterols, stigmasterol and campesterol provide raw materials for steroid intermediates and drugs).

Several methods have been developed to extract these valuable compounds. In the case of the carotenoids, the known methods can be classified as follows:-

- (i) Extraction by saponification e.g. British Patent 567,682; U.S. Patent 2,460,796; U.S. Patent 2,440,029; U.S. Patent 2,572,467; U.S. Patent 2,652,433
- (ii) Iodine method
- (iii) Urea process
- (iv) Extraction using Fuller's earth or activated carbon, e.g. British Patent 691,924; British Patent 1,563,794; U.S. Patent 2,484,040
- (v) Extraction by selective solvents e.g. U.S. Patent 2,432,021

(vi) Molecular Distillation.

In the saponification method (i), the oil is saponified to give soap, glycerol and a non-saponifiable fraction containing carotenes.

5 In the iodine method (ii), the iodine is added to a solution of palm oil in petroleum ether, an insoluble precipitate of carotene di-iodide is formed. The iodo- compound when treated with sodium thiosulphate however yields iso-carotene or
10 dehydro-carotene which has no biological activity.

With the urea method (iii), the triglycerides are broken down to fatty acids and methyl esters which then form insoluble compounds with urea and thiourea, leaving the carotenoids in the remaining
15 liquid.

Extraction of carotenes using adsorbents has been carried out using Fuller's earth and activated carbon (method iv). However, the extraction of the carotenes from the earth gives oxidised or isomerised
20 products of carotenes. Carotene is concentrated six times in the extract.

Extraction of carotenes by selective solvents (method v) has been carried out using propane or furfural. The carotene is concentrated (three times
25 that of the original oil), in the furfural phase.

By method (vi) carotenes can also be obtained by molecular distillation (10^{-3} - 10^{-4} mm Hg). Fractions collected at 230°C have a carotene content of about five times that of the original oil.

30 None of these methods however have been commercialised because of several difficulties.

According to the present invention there is a method for the isolation of the minor non-glyceride components of palm oil or like vegetable oil
35 containing free fatty acid and non-glyceride components similar to that of palm oil, which method

comprises:

(i) Esterifying the free fatty acid component of the oil with one or more monohydric alcohols to form an esterified oil with a very low free fatty acid content,

(ii) converting the glycerides into monoesters by transesterification employing one or more monohydric alcohols,

(iii) adsorbing the non-glyceride components onto a selective adsorbent to separate said components from the esters of the oil, and

(iv) thereafter desorbing the non-glyceride components from the adsorbent with the use of solvent to recover said components.

The present method used a selective adsorbent for the adsorption of the minor non-glyceride components from esterified palm oil. We have found that this method is possible because, unlike crude palm oil, esterified palm oil possesses suitable physical and chemical properties. Passage of the esterified palm oil with or without solvent through a selective adsorbent allows solid phase extraction or trapping of carotenoids, sterols, tocopherols and tocotrienols.

It is a surprising discovery that solid adsorbents such as alumina or silica gel, or carbon contrary to the expectation of a skilled worker in the art, provide a very satisfactory way of obtaining the minor non-glyceride components which include the carotenes, sterols, tocopherols etc. from the original vegetable oils.

In a typical extraction of carotenes from palm esters (e.g. methyl esters) prepared in accordance with British Patent Specification 2148897A, passage through bonded phase silica gel provides a recovery of 70% of the available carotenes in the form of a

concentrate. Extraction is possible in the presence of alcohols (e.g. methanol, ethanol etc.) from which the esters have been prepared. In a typical extraction of sterols, tocopherols and tocotrienols, palm esters are passed through suitable adsorbents such as activated alumina and silica gel where they are selectively adsorbed and later desorbed using suitable solvents. Purification to pure components can be carried out using conventional chromatographic techniques.

The present method allows for the recovery from palm oil of several valuable minor components the value of which can surpass that of the oil. The industrial preparation of palm esters for oleochemicals, detergents, palm diesel, etc. opens up an important avenue for the recovery of these minor components.

Following is a description by way of example of the recovery of carotenoids, tocopherols, tocotrienols and sterols by reverse phase (C18) silica gel carbon and alumina adsorbents.

Example 1

Crude palm oil methyl ester was dissolved in methanol (30 ml) and the mixture was introduced into a glass column packed with C₁₈ reverse phase (15g) the packing having a height of 20 cm and diameter of 1.8 cm. The ester eluted first and was collected and pumped as fraction W₁. More methanol was introduced into the column to elute out as much ester as possible until carotenoid was about to be eluted out and this was collected and pumped as fraction W₂. Hexane and methanol (98:2 v/v) or chloroform was used to elute out the carotenoid and this was collected as fraction W₃. Occasionally the column was then cleaned up once with chloroform (40 ml)

giving rise to fraction W_4 . The column was then soaked in methanol for further use. A chromatographic separation was carried out under a nitrogen atmosphere and the recovery of carotenes was determined at 446 nm. The results are shown in Table 1.

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Table 1 Recovery of Carotenoids from Methyl Esters of Neutralised

Palm Oil using C₁₈ Reverse Phase Adsorbent (I)**

Methyl Esters/g	Solvents used as Eluent*/ml				Collected Fractions/g				Recovery of Carotenoids /%
	A	B	C	D	W ₁	W ₂	W ₃	W ₄	
15.19	30	30	15#	40	8.01	4.76	1.76	0.66	66@
15.02	30	45	55#	15	7.98	4.79	1.86	0.0098	90
15.02	30	30	55#	50##	8.49	5.34	1.17	0.0079	63.9
15.01	30	45	45	40	8.42	5.62	0.94	0.0022	95
15.02	30	45	45	40	0.75	11.62	2.36	0.0034	95
8.01	30	190	45	40	1.79	6.08	0.07	0.0597	81

* Consecutive solvents used as eluent were: A = MeOH (use for dissolving ME);

B = MeOH; C = n-hexane:MeOH (98:2 v/v); D = CHCl₃.

** Weight of adsorbent = 15 g.

Solvent used was chloroform.

Solvent used was ethanol (95%).

@ This value is based on the 3rd and 4th fractions.

Example 2

The procedure of Example 1 was repeated except that different amounts of starting materials were used, i.e. methyl esters had first been eluted and cleaned up by passing through an alumina column. The results are shown in Table 2.

Example 3

The procedure of Example 1 was repeated except that instead of methanol, ethanol was used as eluent and with different amounts of starting materials. The results are shown in Table 3.

Example 4

5 Recovery of carotenoids, tocopherols, tocotrienols, and sterols from transesterified NPO was carried out by adsorption onto C₁₈ reverse phase SiO₂ followed by alumina.

20 Neutralised palm oil methyl ester (15g), which was cleaned by passing through Kieselguhr (70-230 Mesh ASTM), was dissolved in methanol (30 ml) and the mixture was introduced into the glass column packed with C₁₈ reverse phase (15 g, 20 cm height, 1.8 cm diameter). The eluted ester was collected as
25 fraction 1 (9.20 g). Another 45ml of methanol were introduced into the column to elute out as much ester as possible until carotenoids were about to be eluted out and these were collected as fractions 2 and 3
30 (3.67 g and 1.91 g respectively). 45 ml of hexane and methanol (98:2 v/v) were used to elute out the

Table 2 Recovery of Carotenoids from Methyl Esters of Neutralised

Palm Oil using C₁₈ Reverse Phase as Adsorbent (II)**

Methyl Ester ^g /g	Solvents used as Eluent ^g /ml			Collected Fractions/g				Recovery of Carotenoids /%
	A	B	C D	W ₁	W ₂	W ₃	W ₄	
15.00	30	30	15# 15	8.30	5.04	1.56	0.0409	74.7
3.76	30	30	45 30	0.17	2.44	1.03	0.1039	80.6
2.10	30	30	45 30	0.05	1.79	0.23	0.0303	39.2
2.01	30	30	45 30	-	1.90	0.13	0.0150	26
1.04	30	30	45 30	0.01	0.91	0.11	0.0144	4

* Consecutive solvents used as eluent were: A = MeOH (use for dissolving ME);

B = MeOH; C = n-hexane:MeOH (98:2 v/v); D = CHCl₃.

** Weight of adsorbent = 15 g.

Solvent used was chloroform.

@ These methyl esters samples had first been eluted through an alumina column.

Table 3 Recovery of Carotenoids from Methyl Esters of Neutralised

Palm Oil using C₁₈ Reverse Phase as Adsorbent (III)**

Methyl Esters/g	Solvents used as Eluent*/ml				Collected Fractions/g				Recovery of Carotenoids /%
	A	B	C	D	W ₁	W ₂	W ₃	W ₄	
15.02	30	55	55	-	0.17	14.75	-	-	all
8.01	30	60	45	30	0.03	7.59	0.12	-	92.3
6.00	30	100	55	40	3.00	2.80	0.10	0.1003	93.6
3.75	30	30	65	30	0.23	2.76	0.67	0.0381	87

* Consecutive solvents used as eluent were: A = 95% EtOH (use for dissolving ME); B = 95% EtOH; C = n-hexane:EtOH (98:2 v/v); D = CHCl₃.

** Weight of adsorbent = 15 g.

Solvent used was chloroform.

@ All carotenoids had been eluted out together with methyl esters in fraction W₂.

carotenoids and this was collected as fraction 4 (0.1785 g). Chromatography separation was carried out under a nitrogen atmosphere. The percentage recoveries of carotenoids (quantified by uv/visible spectrophotometry), tocopherols and tocotrienols (quantified by GLC) of each of the four fractions above are tabulated in Table 4.

Fractions 1, 2 and 3 (total 12.84 g) in which most of the tocopherols, tocotrienols and sterols were found were then combined and eluted into a glass column packed with neutral alumina (1.43 g; ratio of methyl ester:adsorbent, 9:1 w/w). The height of the packing material was 4.5 cm and the diameter of the column was 0.8 cm. The methyl ester eluted was collected as fraction 1 (11.75g). n-Hexane (2 x 12.9ml) was introduced into the column to clean up as much methyl ester as possible and this was collected as fraction 2 (0.90 g). Finally, chloroform (4 x 8.6 ml)-was used to recover the sterols, tocopherols and tocotrienols from alumina and this was collected as fraction 3. Chromatography separation was carried out under nitrogen atmosphere. Percentage recoveries of tocopherols and tocotrienols, and sterols in each of the above three fractions were worked out and the results are shown in Table 5.

Example 5

The recovery of carotenoids, tocopherols and tocotrienols, and sterols was performed by adsorption onto alumina followed by C_{18} reverse phase silica gel.

Neutralised palm oil methyl esters (90 g) was cleaned up by filtering through Kieselguhr and then eluted into a glass column packed with alumina (neutral, 10 g, 3.5 cm height and 2.5 cm diameter). The eluted methyl esters were collected as fraction 1

Table 4 Recovery of Carotenoids, Tocopherols and Tocotrienols, and Sterols from Methyl Esters of Neutralised Palm Oil using C₁₈ Reverse Phase Silica Gel

Fraction	Recovery*/%		
	Carotenoids	Tocopherols and Tocotrienols	Sterols
1	5.2	61.7 (556)	9
2	3.5	19.9 (450)	8.3
3	6.3	11.9 (516)	6.3
4	67.4	2.3 (1045)	ND#

* Recovery in ppm is bracketed.

ND = Not Detectable.

Table 5 Recovery of Tocopherols, Tocotrienols and Sterols from Methyl Esters of Neutralised Palm Oil using Alumina as Adsorbent (I)*

Fraction	Recovery#/%	
	Tocopherols & Tocotrienols	Sterols
1	79.4 (366)	30.1
2	5.8 (347)	18.9
3	2.6 (1510)	15.4

* The methyl esters used has first been eluted through the C₁₈ reverse phase column to remove carotenoids as shown in Table 4

Recovery in ppm is bracketed.

(82.23 g). n-Hexane (210 ml) was then introduced into the column to clean up as much methyl esters as possible and this was collected as fraction 2 (6.81 g). Finally, chloroform (240 ml) was used to recover the adsorbed components including tocopherols and tocotrienols, and sterols from the starting material used (i.e. neutralised palm oil methyl esters) in the 3 fractions collected is shown in Table 6.

15.3 g of eluted methyl esters from fraction 1 above was then dissolved in methanol (30 ml) and the mixture was introduced into the glass column packed with C_{18} reverse phase SiO_2 (15 g; 20 cm height, 1.8 cm diameter). The ester eluted was collected as fraction 1 (9.04 g). Another 45 ml of methanol was introduced into the column to elute out as much ester as possible before the carotenoids were eluted out and this was collected as fraction 2 (5.83 g). n-Hexane and methanol (98:2, 45 ml) were used to elute out the carotenoids and collected as fraction 3 (0.32 g). The column was then cleaned up once with chloroform (40 ml) and then soaked in methanol for further use. The chromatography was carried out under nitrogen atmosphere. The percentage recoveries of carotenoids, tocopherols and tocotrienols, and sterols of each of the three fractions above were worked out and tabulated in Table 7.

Table 6 Recovery of Tocopherols, Tocotrienols and Sterols from Methyl Esters of Neutralised Palm Oil using Alumina as Adsorbent (II)

Fraction	Recovery//%	
	Tocopherols & Tocotrienols	Sterols
1*	77.8 (357)	72.5
2	10.4 (577)	13.2
3	5.7 (3315)	6.8

* A portion of methyl esters eluted was passed through C₁₈ reverse phase to recover carotenoids as shown in Table 7.

Recovery in ppm is bracketed.

Table 7 Recovery of Carotenoids, Tocopherols and Tocotrienols, and Sterols from Methyl Esters of Neutralised Palm Oil using C₁₈ Reverse Phase as Adsorbent

Fraction	Recovery#/%		
	Carotenoids	Tocopherols and Tocotrienols	Sterols
1	6.04	71.6 (423)	42.5
2	9.72	33.7 (316)	12.3
3	89.06	1.7 (290)	ND

* The methyl esters used has first been eluted through the alumina column as shown in Table 6.

The percentage recovery was based on the starting material used in the column. Recovery in ppm is bracketed.

ND = Not Detectable.

It is understood that in place of the methyl alcohol used to produce the above described methyl esters any of the branched or straight chain alcohol having from 1 to 6 carbon atoms may be used, although methyl alcohol is preferred.

Table 8 Adsorption and Extraction of Carotenoids of Methyl Esters of Crude Palm Oil using Activated Carbon - Continuous Column Extraction*

Experiment	Adsorption of Carotenoids/%	Recovery of Carotenoids/%
1#	79	49
2##	88	50.6

* The following conditions were used:- weight of methyl esters = 5 g; weight of carbon = 1 g; ratio of methyl esters to carbon = 5:1; weight of butylated hydroxytoluene (BHT) = 0.01 g; adsorption was done at 28-30°C; percentage recovery of carotenoids from carbon was from toluene fraction only.

18 ml of petroleum ether b.p. 60-80°C, 88 ml of toluene and 38 ml of toluene/ethanol (3:1 v/v) successively were used as eluent; all solvents were at 28-30°C.

10 ml of n-hexane (of which 5 ml was used to dissolve methyl esters), 43 ml of toluene and 17 ml of toluene/ethanol were used as eluent; both toluene and toluene/ethanol were pre-warmed to 40°C before use.

Table 9 Adsorption and Extraction of Carotenoids of Methyl Esters of Crude Palm Oil using Activated Carbon - Batchwise Extraction*

Exp.	Activated Carbon	Solvent for Recovery/ml	Adsorption of Carotenoids/%	Recovery of Carotenoids/%	Remarks
1	Carbon S511	CH ₂ Cl ₂ ; 120	25.2	3.8	Untreated carbon; Carbon pH = 11.0
2	Norit OL	CH ₂ Cl ₂ ; 120	61.2	3.3	Untreated carbon
3	Carbon S511	CH ₂ Cl ₂ ; 120	70.3	3	Carbon was warmed and vacuum pumped dry before use
4	Carbon S511	CH ₂ Cl ₂ ; 120	73.5	2.4	Antioxidant hydroquinone; carbon was vacuum pumped dry before use.
5	Carbon S511	CH ₂ Cl ₂ ; 120	66.5	10.3	Carbon - treated with EtOH, HCl & Na ₂ CO ₃ ; activated at 300°C; pumped dry at 200°C; pH = 10.1
6#	Carbon S511	Toluene; 60	61.9	26.1	Carbon was pumped at 250°C for 2 hr before use.

* The following conditions were used: weight of methyl esters = 20 g; weight of carbon = 4 g; methyl esters:carbon = 5:1; adsorption of material onto carbon was done at 28-30°C; recovery of carotenoids from carbon was carried out using a Soxhlet extractor with solvent.

Recovery of carotenoids from carbon was done by soaking the carbon in toluene.

CLAIMS

1. A method for the isolation of the minor
5 non-glyceride components of palm oil or like
vegetable oil containing free fatty acid and
non-glyceride components similar to that of palm oil,
which method comprises:

(i) esterifying the free fatty acid
10 component of the oil with one or more monohydric
alcohols to form an esterified oil with a very low
free fatty acid content,

(ii) converting the glycerides into monoesters
by transesterification employing one or more
15 monohydric alcohols,

(iii) adsorbing the non-glyceride components
onto a selective adsorbent to separate said
components from the esters of the oil, and

(iv) thereafter desorbing the non-glyceride
20 components from the adsorbent with the use of solvent
to recover said components.

2. A method as claimed in claim 1 wherein
the adsorbent is activated alumina carbon or silica gel,
25 preferably reverse phase (particularly C 18) silica
gel.

3. A method as claimed in claim 1 or claim 2
wherein the non-glyceride components obtained from
30 step (iii) are separated into sterols, tocopherols,
tocotrienols and carotenes by a chromatographic
technique, or wherein the recovered minor component is only
carotene by using carbon adsorbent.

4. A method as claimed in any one of the
35 preceding claims wherein the esterification of step
(i) is carried out employing (a) a solid alkali

metal bisulphate or (b) a sulphate acid
strongly-acidic ion-exchange resin as a catalyst and
the transesterification of step (ii) is carried out
employing a basic catalyst or both the esterification
5 and transesterification are carried out using an
enzyme e.g. candida rugosa.

5. A method as claimed in any one of the
preceding claims wherein the oil which is esterified
10 in step (i) is a palm oil or a palm oil fraction.

6. A method as claimed in any one of the
preceding claims wherein the carboxylic acid is
esterified and/or the glycerides are transesterified
15 with one or more C_1 to C_3 alcohols, preferably
methanol.

7. A method as claimed in any one of
claims 4 to 6 wherein there is employed from 1 to 20%
20 by weight of catalyst, based upon the weight of the
free fatty carboxylic acid.

8. A method for the recovery of carotenes
from esterified palm oil by employing a C 18 reverse
25 phase silica gel as adsorbent using two combinations
of solvents as consecutive eluents as follows :

(i) Methanol, n-hexane : methanol (98 : 2
v/v) and $CHCl_3$
(ii) Ethanol, n-hexane : 95% EtOH (98 : 2 v/v)
30 and $CHCl_3$.

9. A method as claimed in claim 9 in which
the recovery of carotenes from esterified palm oil is
at least 95% with a ratio of methyl ester to
35 adsorbent of 1 : 1 (w/w) on the recovery of carotenes
from esterified palm oil is at least 92.3% when the

ratio of methyl esters to adsorbent is 0.5 : 1 (w/w).

5 10. A method for the recovery of carotenes from esterified palm oil by employing activated carbon as adsorbent and using aromatic solvent such as toluene or aliphatic solvent such as dichloromethane or ethanol for desorbing carotenes from the carbon.

10 11. A method for the isolation of the minor non-glyceride components of palm oil or the like substantially as hereinbefore described in any one of the examples.

 12. A non-glyceride component of palm oil or the like when obtained using a process as claimed in any one of claims 1 to 11.

15 13. Sterols, tocopherols, tocotrienols and carotenes when obtained from the component of claim 12.

